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Abstract

Many species of insects display dispersing and non-dispersing morphs. Among these, aphids are one of the best examples of taxa that have evolved specialized morphs for dispersal versus reproduction. The dispersing morphs typically produce a full set of wings as well as a sensory and reproductive physiology that is adapted to flight and

reproducing in a new location. In contrast, the non-dispersing morphs are wingless and show adaptations to maximize fecundity.

In this review we provide an overview of the major features of the aphid wing dimorphism. We first provide a description of the wing dimorphism and an overview of its phylogenetic distribution. Second, we review what is known about the mechanisms underlying the wing dimorphism. Finally, we discuss evolutionary aspects of the dimorphism.

We distinguish between environmentally induced dimorphism, which is called polyphenism, and genetically determined dimorphism, which is called polymorphism. In addition, natural populations display genetic variation for the environmental induction of winged morphs. This is, in effect, genetic polymorphism for a polyphenism.

Aphid biology

Aphids are small, soft-bodied insects of the order Hemiptera that feed on the fluid in plant phloem. Aphids exhibit complex life cycles. Approximately 10% of species alternate between a primary (usually woody) host plant and a secondary (herbaceous) host plant. Non host-alternating species are usually monophagous or feed on a range of related host plants (BLACKMAN and EASTOP 1994). Aphids display a high reproductive rate due to three peculiarities of their reproductive biology. First, during the spring and summer months, female aphids reproduce parthenogenetically, obviating the need for males. Second, during these parthenogenetic generations, the embryos initiate

development immediately after the budding of the oocyte from the germarium and are born as fully developed first-instar nymphs (i.e. they are viviparous). Finally, the oldest embryos also contain embryos, so that adult parthenogenetic aphids carry not only their daughters but also some of their granddaughters within them. During the fall, declining daily photoperiod and temperature induce the development of sexual females and males. These sexual aphids mate and females produce yolk-rich eggs that undergo diapause to survive the winter (i.e. they are oviparous).

Differences between winged and wingless aphid morphs

The winged and wingless phenotypes in aphids differ in a range of morphological, physiological, life history and behavioural features. Besides having wings and functional flight muscles, the fully winged morph exhibits heavier sclerotization of head and thorax, more fully developed compound eyes, ocelli, longer antennae, more rhinaria, and sometimes larger siphunculi and cauda (KALMUS 1945; KAWADA 1987; KRING 1977; MIYAZAKI 1987). Most of these differences reflect the different life styles of the two phenotypes, as the winged morphs are equipped with an elaborate sensory system for flight and host plant location. Consistent with this theme, winged forms are also more resistant to starvation (HAZELL *et al.* 2005; TSUJI and KAWADA 1987b).

The morphological differences between winged and wingless phenotypes usually correlate with differences in life history. In general, the winged phenotype differs from the wingless phenotype by showing longer nymphal development, longer pre-reproductive adult period, longer reproductive period, lower offspring production, and

prolonged longevity (CAMPBELL and MACKAUER 1977; MACKAY and WELLINGTON 1975; NODA 1960; TSUJI and KAWADA 1987b; TSUMUKI *et al.* 1990). In addition, in response to shortened photoperiod, winged females tend to produce mainly sexual females whereas wingless females produce both sexual females and males (MACKAY *et al.* 1983; NUNES and HARDIE 1996).

Phylogenetic distribution of wing dimorphisms

Phylogenetic evidence indicates that the wingless form in aphids has been secondarily derived, as in most other insects. Within the Aphididae – comprising more than 95% of all 4400 extant aphid species – there is extensive variation in the occurrence of winged and wingless morphs at different stages of the life cycle. Many species of the more primitive taxa, such as the Calaphidinae, produce only winged parthenogenetic females (HEIE 1982; HILLE RIS LAMBERS 1947; HILLE RIS LAMBERS 1966). In some of these species, however, some winged females may differ in their flight capability or colonies may display variation in wing length (DIXON 1972; HEIE 1982; HEIKINHEIMO 1987; HILLE RIS LAMBERS and VAN DEN BOSCH 1964; HILLE RIS LAMBERS 1966). In *Drepanosiphum dixoni*, for example, all parthenogenetic females develop wings, yet some individuals lack indirect flight muscles and are therefore not capable of flight (DIXON 1972). In other calaphidine species (e.g. *Symydobius oblongus*) the parthenogenetic females show consistent differences in wing length, and the short-

winged females do not fly (HEIKINHEIMO 1987). Most species of the Aphididae, however, produce fully winged and completely wingless parthenogenetic females.

The diverse dispersal strategies of aphids appear to have evolved in association with two dominant ecological factors: host plant alternation and type of host plant. In host-alternating species, the morphs migrating between the primary and secondary hosts are always winged, whereas both winged and wingless females frequently occur during the parthenogenetic generations on the summer host for both host-alternating and non host-alternating species (BLACKMAN and EASTOP 1994). Certain host plant characteristics, such as host plant persistence, may also correlate with the propensity to produce predominantly winged or wingless parthenogenetic females. Ephemeral host plants, including annual herbaceous hosts, can be exploited only temporarily and dispersal to new or more persistent hosts must take place at some point during the life cycle. Consistent with this idea, species feeding on large or perennial host plants exhibit a lower incidence of winged morph production (GROETERS 1989). An exception are tree-dwelling aphid species, which often produce exclusively winged females compared to species feeding on herbaceous plants, possibly because flight allows aphids to find a suitable feeding location in architecturally complex trees (WALOFF 1983).

In most species the sexual females are wingless, with some exceptions in more primitive taxa. Sexual males of Eriosomatinae, Anoeciinae, Hormaphidinae and some Lachninae are wingless. In most other taxa males are winged; for example, the males of all host-alternating aphidines are invariably winged (BLACKMAN and EASTOP 1994; BLACKMAN and EASTOP 2000). In about 10% of European species, however, both winged and wingless males have been recorded (SMITH and MACKAY 1989). In the few species

that have been examined in detail, this dimorphism is apparently caused by a genetic polymorphism (HILLE RIS LAMBERS 1966; MÜLLER 1969; SMITH and MACKAY 1989).

Mechanisms

Environmental cues

The environmental conditions affecting the production of winged versus wingless morphs have been studied intensively (HILLE RIS LAMBERS 1966; KUNKEL and KLOFT 1974; LEES 1966; MITTLER and SUTHERLAND 1969; MÜLLER *et al.* 2001). Here we briefly summarize the conditions known to cue production of the winged morph, focusing on parthenogenetic females.

Density (tactile stimulation)

Density-dependent regulation of alternative dispersal phenotypes is common in aphids and other insects. Increased aphid density triggers wing formation in many species and in some species a small increase in density is sufficient (BONNEMAISON 1951; JOHNSON 1965; LEES 1967; SHAW 1970a; SUTHERLAND 1969a; SUTHERLAND 1969b). The proximate mechanism mediating these environmental conditions appears to be increased tactile stimulation among aphids (JOHNSON 1965). However, it is possible that chemical cues play an additional minor role (KUNERT and WEISSER 2005). While the location of the tactile receptors is not known, in some species perception is mediated mainly by the antennae (JOHNSON 1965; LEES 1967; SUTHERLAND 1969a).

Host plant quality (nutrition)

For a given aphid clone, variation in winged morph production correlates with variation in host plant species (VERESCHAGINA and SHAPOSHNIKOV 1998) and with changes in host plant quality or host plant age (SUTHERLAND 1969b). However, a review by Müller et al. (MÜLLER *et al.* 2001) showed that more than half of 38 examined studies in twelve different aphid species did not confirm the hypothesis that poor nutrition is responsible for an increase in winged morph production. In many of the earlier studies, the reported host plant effect on winged morph production was likely due to the effect of the host plant on the number of physical contacts between aphids (MÜLLER *et al.* 2001). Nevertheless, in some species there does appear to be evidence that a decrease in plant quality alone can trigger wing induction in some species (MÜLLER *et al.* 2001).

Interspecific interactions

Interactions among different aphid species that aggregate on the same host plant can lead to an increased production of winged individuals (LAMB and MACKAY 1987), but this is likely to reflect a density-dependent response. In contrast, the mere presence of particular natural enemies may elicit an increase in winged morph production in the pea aphid, *Acyrthosiphon pisum* (DIXON and AGARWALA 1999; KUNERT and WEISSER 2003; SLOGGETT and WEISSER 2002; WEISSER *et al.* 1999). (Parasitization may also directly affect wing development, see below). The induction of wing development seems to result from increased tactile stimulation triggered by either predator avoidance behavior or from

the release of aphid alarm pheromone (KUNERT *et al.* 2005). The presence of ants (which provide some protection for aphids against predators) seems to inhibit the production of winged individuals (EL-ZIADY and KENNEDY 1956; KLEINJAN and MITTLER 1975). Aphid or plant pathogens (e.g. fungi, viruses) and the facultative endosymbionts may also affect wing induction (LEONARDO and MONDOR 2006; MÜLLER *et al.* 2001).

Abiotic factors

Several other factors, in particular temperature, may influence wing production either directly or indirectly via the host plant (JOHNSON and BIRKS 1960; KENTEN 1955; LIU 1994; SCHAEFERS and JUDGE 1971; WHITE 1946). Most studies have reported a decline in winged morph production as temperature increases (MÜLLER *et al.* 2001). Photoperiod may be responsible for wing induction of parthenogenetic females in clones that do not undergo sexual reproduction (LEES 1966).

Maternal effects

In addition to environmental factors, several maternal and transgenerational influences affect winged morph determination. In many species where wing determination occurs prenatally, winged adults rarely or never produce winged offspring even when exposed to wing-inducing stimuli (LEES 1961; SUTHERLAND 1970). Similarly, early born progeny descending from winged mothers themselves exhibit a decreased production of winged morphs (MACKAY and WELLINGTON 1977). In contrast, early born (wingless) progeny derived from wingless mothers respond strongly to wing-inducing

stimuli (MACKAY and LAMB 1979; MACKAY and WELLINGTON 1977). Grand-maternal phenotype, maternal phenotype, and age therefore all affect and modulate the response to wing-inducing environmental conditions.

Development

The development of alternative phenotypes has been examined in several aphid species using histological methods (GANASSI *et al.* 2005; JOHNSON and BIRKS 1960; KITZMILLER 1951; SHULL 1938; TSUJI and KAWADA 1987a; WHITE 1946). Wing development appears to be the default developmental pathway and the wingless phenotype develops by diversion from this developmental pathway during prenatal or postnatal development. For example, JOHNSON and BIRKS (1960) examined a large number of fully developed embryos and first instar nymphs of *Aphis craccivora* and found wing anlagen in all of them, irrespective of whether or not they were destined to develop into winged adults. In this species wing anlagen first appear as hypodermal thickenings shortly before the embryonic moult (JOHNSON 1958a), which takes place about one day before birth. The wing buds increase in size until the embryo is born. In nymphs destined to be wingless, the anlagen cease development at this stage. A similar scenario has been described in the pea aphid where all embryos, first-instar nymphs and second-instar nymphs exhibit wing buds, which subsequently degenerate in the developing wingless morph (TSUJI and KAWADA 1987a).

Intermediates between winged and wingless phenotypes can occur, although these do not seem to be common. Such intermediates may be wingless but show some

morphological features of the winged morph or they may be winged without having flight muscles and show morphological characteristics of the wingless morph. In the case of *Aphis fabae* and other species, these intermediates can be induced when wing-inducing stimuli are removed at different time points of nymphal development (SHAW 1970b). Furthermore, parasitoids that lay their eggs in adult aphids affect the wing development of offspring. Such offspring very frequently show an intermediate winged-wingless phenotype and wing development is inhibited in presumptive winged individuals (CHRISTIANSEN-WENIGER and HARDIE 1998; CHRISTIANSEN-WENIGER and HARDIE 2000; JOHNSON 1958b; JOHNSON 1959).

The role of hormones in determining the wing polyphenism

Juvenile hormone

The observation that wingless adults and nymphs are morphologically similar led early workers to suggest that high titers of juvenile hormone (JH) induce the wingless state by promoting the retention of juvenile features in adults (JOHNSON 1959; KENNEDY and STROYAN 1959; LAMB 1956). Naturally occurring JHs, as well as crude JH-containing extracts, JH precursors, and JH analogs, have all been tested for their ability to inhibit the induction of winged morphs when administered both prenatally to mothers and postnatally to nymphs.

These studies have yielded disparate and sometimes conflicting results (HARDIE and LEES 1985), in part due to mistaking mere juvenilization by JH for authentic apterization (LEES 1977), but also because of differences in species, dosages, means of

administration, and experimental design. One clear result concerns the migratory winged morphs that produce sexual females, which in certain species (e.g., *A. fabae*) can be induced by short days. Both long days and natural JHs administered to 1st and early 2nd instar nymphs of these individuals cause them to develop as wingless morphs (HARDIE 1980; HARDIE 1981). This appears, however, to be a special case – unrelated to the summer wing polyphenisms – in which JH is able to mimic the effect of long days. Indeed, the effects of both long days and JH can be cancelled out by crowding, suggesting that something other than JH mediates the density-cued wing polyphenism (HARDIE 1980).

Attempts to correlate the activity of the organ that secretes JH, the corpus allatum, with winglessness have also yielded conflicting results. Several studies showed that 3rd and 4th instar nymphs that do not possess wing buds possess larger corpora allata, either by volume or nuclei diameter (ELLIOT 1975; WHITE 1965; WHITE 1968a; WHITE 1971). In at least one other species, however, this correlation does not hold (LECKSTEIN 1976; LECKSTEIN and LLEWELLYN 1975) and the working assumption that volume or nuclei diameter are suitable proxies for either corpus allatum secretory activity or JH titer may be invalid (HARDIE and LEES 1985). Attempts to measure JH directly have detected JH III at very low levels in *Megoura viciae* (HARDIE *et al.* 1985), but no study has successfully correlated JH titers with the production of wingless morphs.

Precocenes

The failure to unequivocally induce or correlate winglessness with JH led to the proposal that the use of anti-JH compounds or experimental destruction of the corpus allatum might break the experimental impasse (HALES 1976). Cells of the corpus allatum are selectively destroyed by the plant-derived precocenes, Precocene I (PI) and Precocene II (PII), as well as the more potent synthetic precocene, Precocene III (PIII) (OHTA and BOWERS 1977). In sensitive species, precocene treatment thus prevents JH synthesis and results in the precocious appearance of adult characteristics in nymphal instars (BROOKS and MCCAFFERY 1990). Consistent with a predicted role for JH in mediating the aphid wing polyphenism, PII applied prenatally can induce the entire suite of characteristics found in the winged morph (DELISLE *et al.* 1983; GAO and HARDIE 1996; HARDIE 1986; HARDIE *et al.* 1996; HARDIE *et al.* 1995; MACKAUER *et al.* 1979; RUP and SOHAL 1989). However, PIII is capable of *inhibiting* the production of winged morphs, at least in the pea aphid (GAO and HARDIE 1996; HARDIE *et al.* 1995).

These contradictory effects of precocenes on winged morph induction are probably not mediated by JH. Although PII is able to induce winged progeny in several species, the majority of studies suggest that it fails to induce precocious development, the classic JH-mediated hallmark of precocenes (DELISLE *et al.* 1983; HARDIE 1986; HARDIE *et al.* 1996; HARDIE *et al.* 1995; MACKAUER *et al.* 1979). Consistent with this result, JH generally fails to reverse the winged morph-inducing effects of PII (GAO and HARDIE 1996; HARDIE 1986; HARDIE *et al.* 1995). Moreover, although the inhibition of winged morph production caused by PIII *is* accompanied by precocious development and destruction of the corpus allatum (HALES and MITTLER 1981; HARDIE 1986; HARDIE *et al.* 1996; HARDIE *et al.* 1995; KAMBHAMPATI *et al.* 1984), the application of JH is

capable of rescuing precocious development without reversing the inhibition of winged morphs (GAO and HARDIE 1996). Together, these observations suggest that PII and PIII exert their effects on the wing polyphenism independently of JH, and that the target mediating the effect of precocene on winged morph production remains unknown.

These results for precocenes, as well as a dearth of clear positive evidence for regulation by JH, leave the question of hormonal regulation of wing induction unanswered. Other than one study reporting a negative result for ecdysterone (APPLEBAUM *et al.* 1975), no other hormonal candidates for mediating the wing polyphenism have been investigated despite the likely requirement, for species showing prenatal induction, that the morph-determining signal must be able to cross the haemolymph. In seeking other candidates, it is perhaps worth keeping in mind one old but telling result for both *Aphis craccivora* and *Megoura viciae*: decapitation of winged morph-producing females leads to the almost immediate and exclusive production of wingless morphs, suggesting that signals either from the brain or the corpus allatum of the mother are likely to induce winged characteristics, not suppress them as the JH model suggests (JOHNSON and BIRKS 1960; LEES 1966).

Evolution

Origin and maintenance of alternative phenotypes

In general, most discrete phenotypic variation seems to arise from continuous phenotypic variation with selection acting against the expression of intermediate phenotypes. The

expression of alternative phenotypes may underlie an actual developmental switch, so that no intermediate phenotypes can be generated. Alternatively, the observed occurrence of discrete phenotypes is due to the discrete occurrence of alternative environmental cues, yet intermediate phenotypes may be produced under certain environmental conditions (NIJHOUT 1999). For aphid wing polyphenisms, both situations may occur depending on the species (see above).

For most organisms displaying alternative phenotypes, neither phenotype exhibits higher fitness. Rather, there is a trade-off, with the relative fitness of the different phenotypes being contingent upon environmental conditions. The evolution and maintenance of alternative phenotypes therefore requires variation in the environment. In aphids and many other wing-dimorphic insects, the winged morph usually has a lower individual fecundity than the wingless morph in the laboratory (see above). The production of these and other alternative phenotypes enables an aphid clone to flexibly specialise for different environments and functions. As aphids produce alternative phenotypes within a clonal unit, there is no genetic conflict among individuals and selection acts to produce an optimal investment in alternative phenotypes for a given clone.

An environmental induction mechanism of alternative phenotypes requires several specific conditions to be met. First, environmental conditions must influence development to generate different phenotypes. Second, the resulting phenotypes must exhibit higher than average fitness in their respective environments. The factors acting as cues may be the same as the selective agents or may be different. Because the developmental environment precedes the selective environment, an environmental cue

must at least be correlated with future selective factors. Environmental control of alternative phenotypes can therefore evolve in organisms living in spatially or temporally variable environments in which cues can be used to reliably predict the future selective environment (MORAN 1992).

Genetic variation for the wing polyphenism

Different clones of the pea aphid, *Acyrthosiphon pisum* (BRAENDLE *et al.* 2005b; HAZELL *et al.* 2005; LOWE and TAYLOR 1964; MARKKULA 1963; WEISSER and BRAENDLE 2001), and other species (BLACKMAN 1979; GROETERS 1989; KVENBERG and JONES 1974; MACGILLIVRAY and ANDERSON 1958) display variation in the propensity to produce winged females, even when exposed to the same environmental conditions. The causes for this genetic variation in the plastic response to environmental cues are not known. Some of this variation may be related to the host plant preferences of aphid clones (MACGILLIVRAY and ANDERSON 1958; WEISSER and BRAENDLE 2001), yet variation is also observed in clones collected from the same host plant species (BRAENDLE *et al.* 2005b).

Male wing polymorphism in the pea aphid

Winged and wingless males of the pea aphid are found both in populations in the ancestral range of Europe and in introduced populations in North America (BLACKMAN and EASTOP 1994; BLACKMAN and EASTOP 2000; CARTIER 1963; HILLE RIS LAMBERS

1966; MEIER 1958; MÜLLER 1962). The male polymorphism is controlled by a single locus on the X chromosome called *aphicarus* (*api)* (BRAENDLE *et al.* 2005a; CAILLAUD *et al.* 2002; SMITH and MACKAY 1989). In aphids, males are haploid for the X chromosome, so one allele of *api* causes winged males and the other causes wingless males. Given that males are produced only for a short period of time in the autumn, a lack of available environmental cues might explain why the male polymorphism relies upon a genetic switch.

Three *api* genotypes segregate in natural populations: clones homozygous for the *api*-winged allele that produce all winged males, clones homozygous for the *api*-wingless allele that produce all wingless males and clones heterozygous for *api* that produce winged and wingless males in equal proportions. Although all three possible *api* genotypes may occur on the same host plant species, several studies suggest that male morph production may correlate with host plant range and persistence (EASTOP 1971; MARKKULA 1963; MEIER 1958; MÜLLER 1962). The precise costs and benefits of producing winged versus wingless males are not known. Only winged males can move long distances to find mates on distant host plants. On the other hand, wingless males develop faster into adults thus may out-compete winged males born on the same host plant. There may therefore be an advantage to producing both winged and wingless males.

Relationship between female wing polyphenism and male wing polymorphism

Since the female wing polyphenism mimics phenotypes generated by the male wing polymorphism, one question is whether the genotype-environment interaction responsible

for the polyphenism involves the same loci that control the polymorphism. We attempted to test this possibility by examining the wing-induction tendencies of the three possible *api* genotypes (BRAENDLE *et al.* 2005b). We derived F_2 clones from a cross between parental clones homozygous for the two *api* alleles, which randomized alleles at loci that were not closely linked to *api*. In every case, clones homozygous for the *api*-winged allele produced few winged females. In contrast, clones heterozygous for *api* or homozygous for the *api*-wingless allele produced comparatively large numbers of winged females. In other words, alleles of the *api* locus producing the male winged morph and unknown loci influencing female wing plasticity are genetically linked in opposite phase. We do not know the extent of the linkage between *api* and the factors contributing to variation in wing polyphenism, but in the extreme they could also represent pleiotropic effects of *api*. One important caveat of this work is that this linkage has been found in progeny derived from a single cross of two wild lines homozygous for the two *api* alleles. Without analyzing a larger sample of clones segregating for the different *api* alleles, we do not yet know if the genetic variation for the female polyphenism and *api* are in linkage disequilibrium in natural populations.

Future Directions

Aphid wing dimorphism illustrates how a single genome can express phenotypic alternatives. The wing polyphenism provides a clear example of adaptive phenotypic plasticity. The co-expression of a wing polymorphism in males, as seen in the pea aphid, provides a rare opportunity to address the interrelation between environmental and genetic induction of alternative phenotypes. This may also help to clarify how genetic and environmental factors interchangeably control the same developmental events, and how evolutionary transitions between polyphenisms and polymorphisms take place. From a practical point of view, the identification of genes involved in the polymorphic control of alternative phenotypes may facilitate the study of polyphenism with a similar set of alternative phenotypes.

Aphid ecology and physiology have been studied intensively during the past few decades but little is known about the genetics and development underlying the expression of alternative phenotypes. Currently, aphids are becoming an increasingly important model system because a large community has begun to develop genomic resources for the pea aphid, *Acyrthosiphon pisum*. These resources will provide novel opportunities for investigating the aphid wing dimorphism and many other features of aphid biology, such as aphid-plant interactions and the relationship between aphids and their endosymbiotic bacteria. The genome of the pea aphid is currently being sequenced at the Human Genome Sequencing Center at Baylor College of Medicine with funds provided by the National Human Genome Research Institute (http://www.hgsc.bcm.tmc.edu/projects/aphid/).

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Figures

Figure 1. The female polyphenism (left) and male genetic polymorphism (right). In both cases, discrete alternative wingless (top) or winged (bottom) morphs are produced.

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